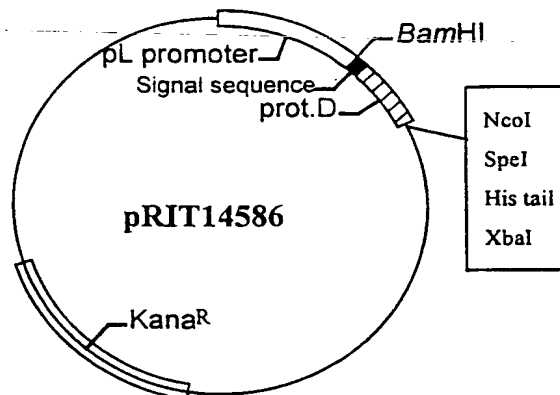


Figure 1: A/ Map of plasmid pRIT14586

B/ Coding sequence of the first 127 amino acids
of protein D and multiple cloning site. The signal
sequence is underlined.

BamHI
 ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTA CTA GCA GGT TGT AGC AGC
 Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser
 CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT
 His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly
 GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT
 Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln Ala
 GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC
 Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His
 TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT
 Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg
 TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA
 Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu
NcoI ACC ATG GCC ACG TGT GAT CAG AGC TCA SpeI ACT AGT GGA CAC CAT CAC CAT CAC CAT TAA TCT AGA XbaI
 Thr Met Ala Thr Cys Asp Gln Ser Ser Thr Ser Gly His His His His His His *

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid sequence of
Figure 1 relates to Seq. ID. No. 6.

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGGKWSKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAA

09509239.032300

TCCCGAGGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVSLSKQPTSQSRGDPTGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAAGTGCCTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAACTGCT
TGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTCATAACA
AAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 13)

^^
MGGKWSKSSVVGWPTVVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTA
CTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSR
GDPTGPKETSGHHHHHH.

E.coli-expressed constructs (fusion constructs)

⇒ LipoD-Nef-HIS

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
 AGCAGCCATTCAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
 GCTCACC GTGGT GCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
 GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
 CGTTTAGTGGTTATTCACGATCACTTTT TAGATGGCTTGACTGATGTTGCGAAAAA
 TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
 GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTT
 GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
 GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
 AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCCGA
 GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCACCATCACCAT
 TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
 GRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGGKW
 SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ LipoD-Nef-Tat-HISDNA sequence (Seq. ID. No. 16)

00509239-032300

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
CGTTTAGTGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGCAAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTT
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTA
GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAATTGCTATTGT
AAAAAGTGTGCTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCC
TATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
CAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG
AAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQQADYLEQDLAMTKD
 GRLVVIHHDHFLDGLTDVAKKFPHRHRKDGRIYVIDFTLKEIQSLEMENFETMGGKW
 SKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFVPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCY
 CKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTG
 PKETSGHHHHHHH.

⇒ ProtD-Nef -HISDNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIAHRGASGYLPEHTLESKALAFQAQADYL
EQDLAMTKDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLK
EIQSLEMTENFETMGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDL
EKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSH
FLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFGW
CYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFDSRLAFH
HVARELHPEYFKNCTSGHHHHHH.

⇒ ProtD-Nef -Tat-HISDNA sequence (Seq. ID. No. 20)

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Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCCTGGTGCTAGCGGTTATTTACCAGAGCATACTGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAA
GGGCTAATTTACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTACACCCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGAT
CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAAT
TGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTA
GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCG
ACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMT
KDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVYRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHT
QGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTN
CYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDP
TGPKETSGHHHHHH.

⇒ Tat-MUTANT-HIS

DNA sequence (Seq. ID. No. 22)

SECRET

Mutated amino-acids in Tat sequence are in bold.

DECLASSIFIED

MCS POLYLINKER: *nef* gene inserted between NcoI and SpeI sites.

Acu II *Nco I* *Spe I* *Eco RI*
TTCGAA.ACC.ATGGCCGCGGACTAGT.GGC.CAC.CAT.CAC.CAT.CAC.CAT.TAA.CCGAATTC

The amino acid sequence of Figure 3 relates to Seq. ID no. 27 and the nucleic acid sequence of Figure 3 relates to Seq. ID. No.26.

Daiichi Silver Staining

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 2: TNH/23 SP eluate (250 ng)
- 3: TNH/23 Purified bulk (250 ng)
- 4: TNH/22 Purified bulk (250 ng)
- 5: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 6: TNH/23 SP eluate (400 ng)
- 7: TNH/23 Purified bulk (400 ng)
- 8: TNH/22 Purified bulk (400 ng)

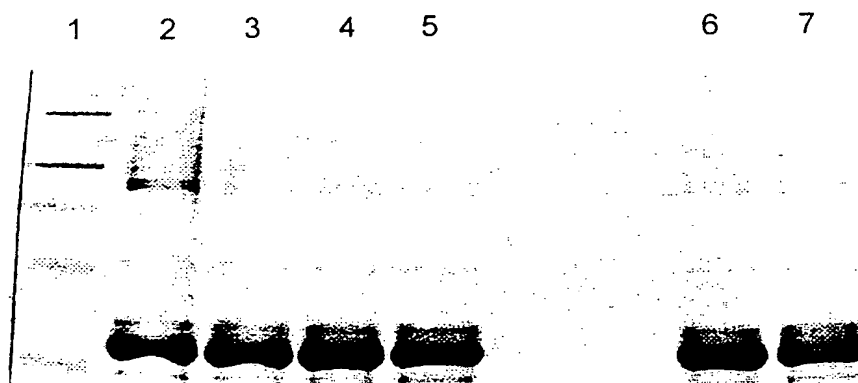
1 2 3 4

1 2 3 4

Blot Tat2

Blot α Nef-Tat (LAS 97340)

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Fig . 5 SDS-PAGE: Nef-Tat-his fusion proteinCoomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
2: TNH/23 SP eluate (4 µg)
3: TNH/23 Superdex200 eluate (4 µg)
4: TNH/23 Purified bulk (4 µg)
5: TNH/22 Purified bulk (4 µg)

6: TNH/23 Purified bulk (4 µg) / non reducing conditions
7: TNH/22 Purified bulk (4 µg) / non reducing conditions

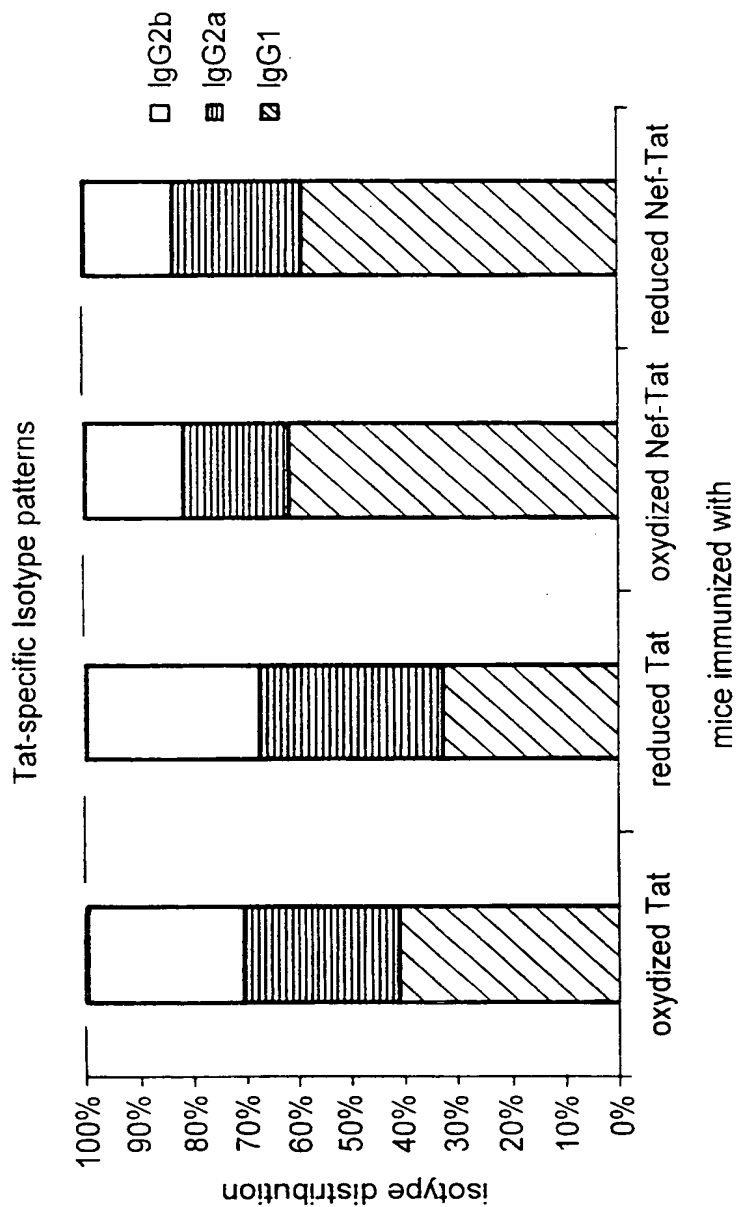
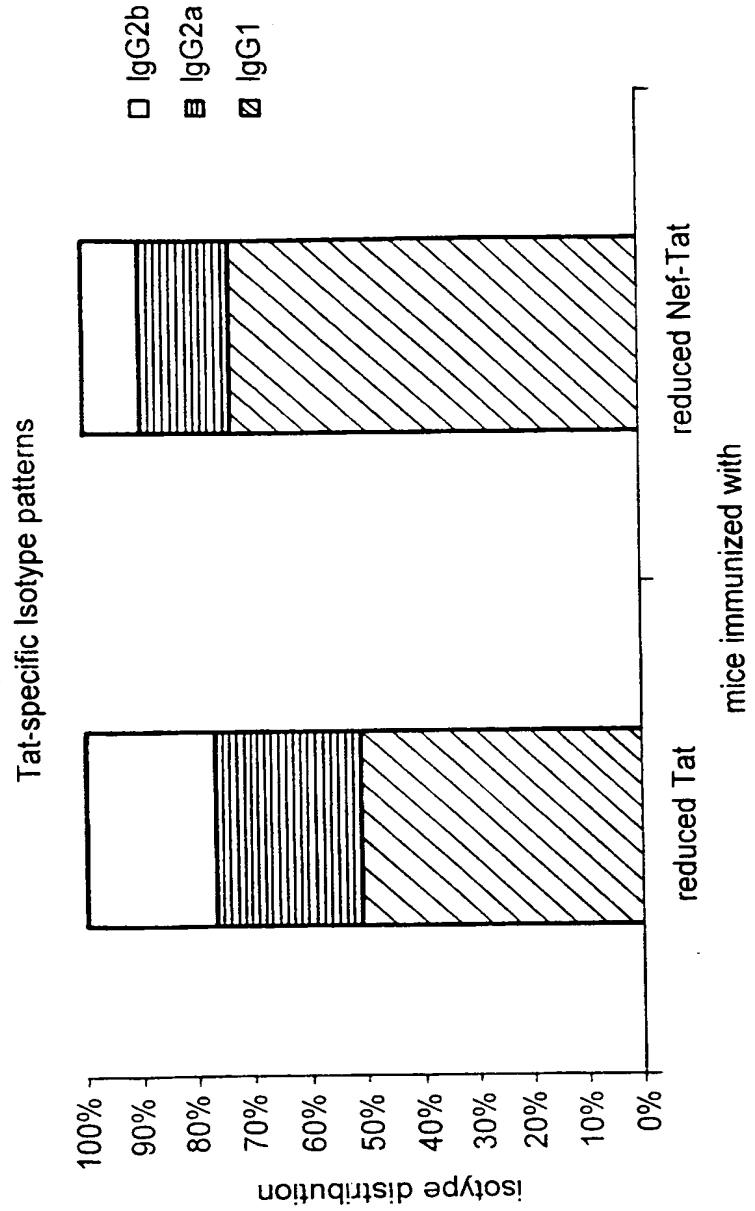


Fig. 6B Tat-specific antibody titers and isotypes

group	immunization	midpoint titers				ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b	
1	reduced Tat	212799	123242	62697	55763	1,966
2	reduced Nef-Tat	75676	84046	18449	11692	4,556
3	adjuvant only	<4000	<4000	<4000	<4000	



002220-62260560

Fig. 7 Antigen-specific lymphoproliferative response of pooled lymph node cells

[3H] Thymidine incorporation in cpm		Data expressed as stimulation index		
		Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat	5µg/ml	140	115	1
	1µg/ml	125	201	1
	0.2µg/ml	92	145	1
reduced Nef-Tat	5µg/ml	146	197	1
	1µg/ml	113	174	0
	0.2µg/ml	84	142	1
medium		1	1	1

	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat	41967	18511	789
	37609	32346	415
	27640	23408	397
reduced Nef-Tat	43882	31694	483
	33865	28094	245
	25079	22891	383
medium	300	161	571

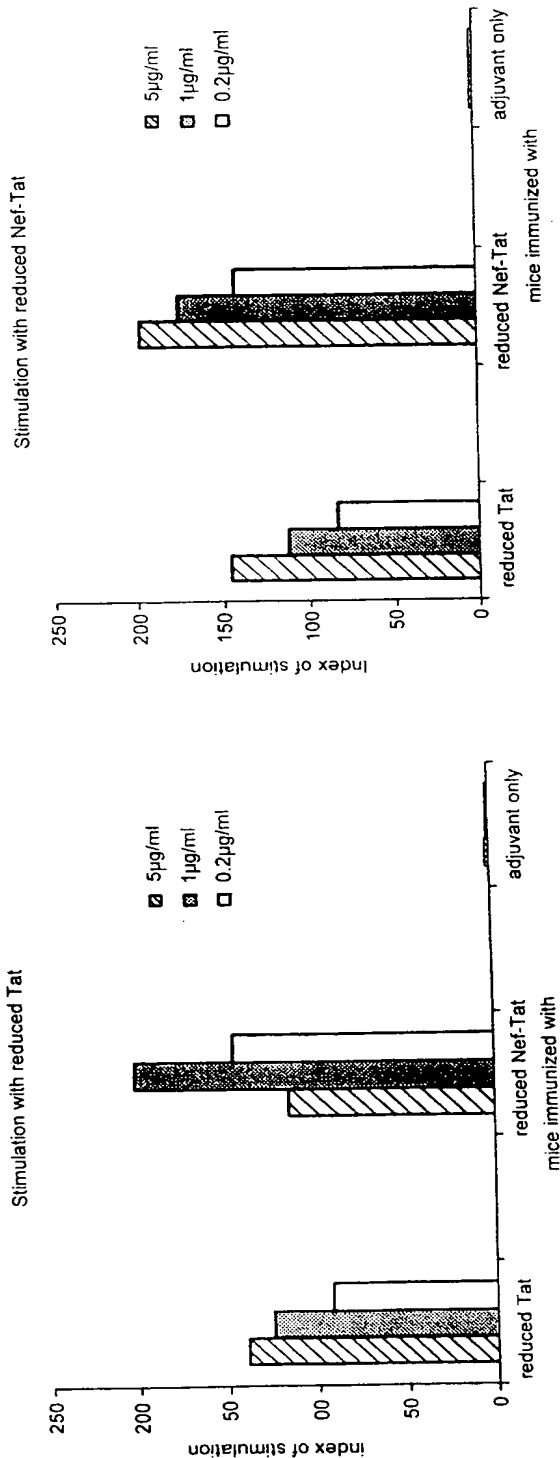


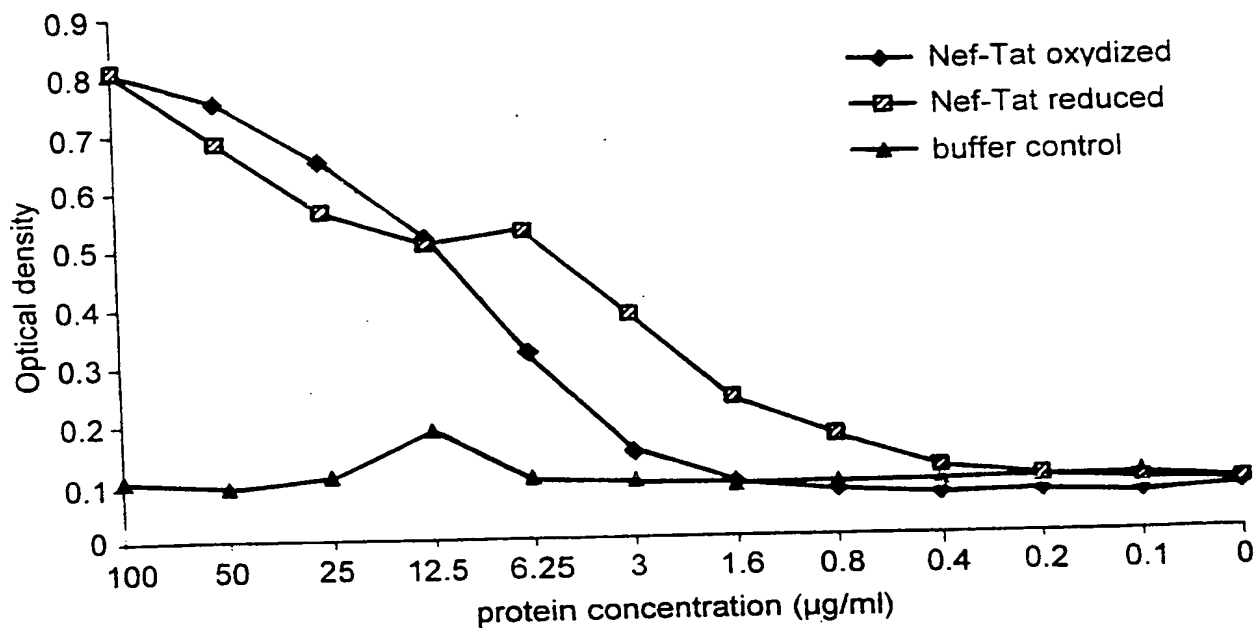
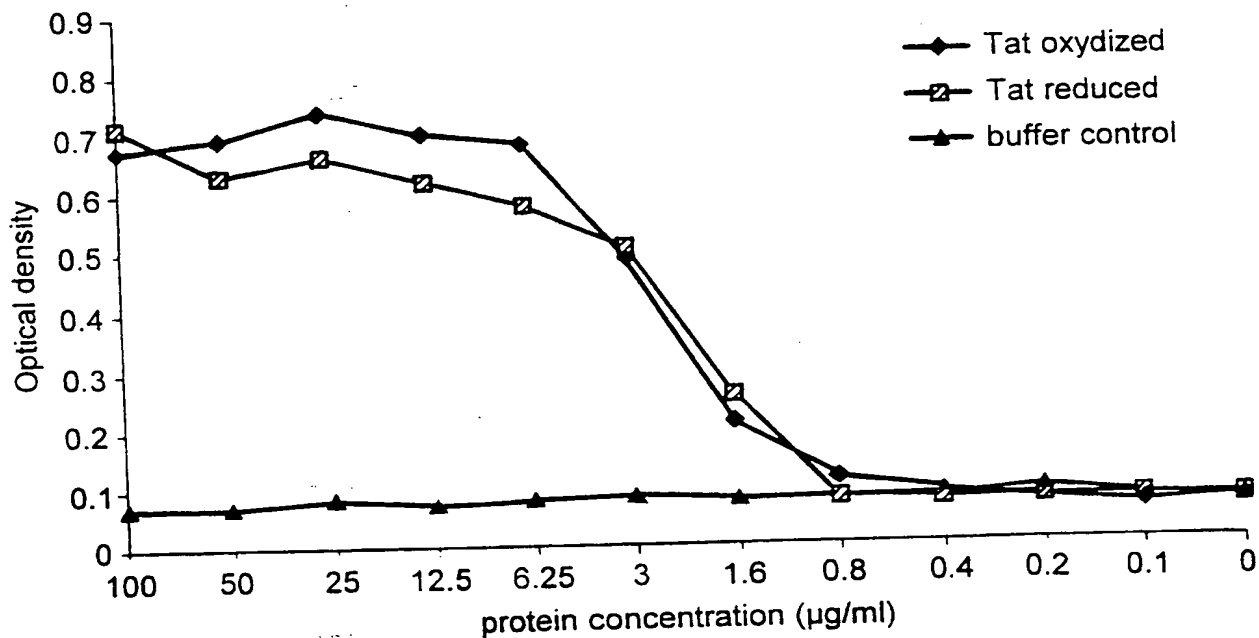
Fig. 8 Cell binding assay

Fig. 9 Inhibition of cell growth